Research Paper
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In*VITRO*Callus Induction and Quantification of Polyphenol from *(Vitisvinifera)* and *(Stevia rebaudiana)*

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Abstract

Medicinal plant researches have been realized in the identification of the germplasm, their multiplication, evaluation of their therapeutic potential and elicitation of the bioactive compound. The*V.vinifera* and *S.rebaudiana* are aphenolic yielding plant belonging to family Vitaceae and Asteraceae. The plant parts possess diverse pharmacological activities including anticancer and antidiabetic property. The present study was carried out for analytical study by performance of TLC from ethanolic extraction. The bioactive secondary metabolites resveratrol and stevioside content of leaf derived by performance of TLC.Solvent system for Thin layer chromatography of stevioside is chloroform: methanol: water and for resveratrol is ethyl acetate: methanol: water. The retardation factor (R_f) for stevioside and resveratrol are 0.63 and 0.87 respectively. The analytical technique revealed that the polyphenolic resveratrol and stevioside content of *in-vivo* leaf extract of *Vitis vinifera* and *Stevia rebaudiana*give R_f value which is similar to standard R_f value.

Keywords:Stevia and Grapes, Ethanolic extraction,Thin layer chromatography, Stevioside and Resveratol

Abbreviations: TLC-Thin layer chromatography

Introduction

Secondary metabolites are essential for plant survive but not necessary for plant growth. *Stevia rebaudiana and V.vinifera*,a God gifted plant having medicinal and commercial in being used all over the world.*S. rebaudiana* is one of the 154 members of genus of Stevia (Pande and khetmalas 2012) and for *V.vinifera* is genus of 79 accepted species of vining plants. *Steviarebaudiana Bertoni*and *Vitis vinifera* is a herbaceous perennial shrub plant belong to the family of Asteraceae and Vitaceae and growing February to April of the year. The native for *S.rebaudiana*of South America particularly Paraguay and Brazil (Anbazhagan et al. 2010) and for *V.vinifera* Iran, Turkey, China, USA, India.. The dry extract from the leaves of *S. rebaudiana*contains sweet diterpene glycosides, flavonoids, water-soluble chlorophylls and xanthophylls, free sugars, amino acids, lipids, essential oils, and trace elements (Komissarenko et al 1994;Esmat and Ferial 2009) and for

*V.vinifera*comprises multipart compounds such as sugars in the form of glucose and fructose, acids like tartaric and malic acid, amino acids, polyphenol, proteins, anthocyanin and flavonals. Stevioside and resveratol is natural polyphinolic compound *isolated* from leaves of Stevia and Grapes. . Stevioside and Resveratol may have beneficial effect on human health. Several reported pharmacological properties for *S.rebaudiana*like antidiabetic, insulinotropic, glucagonestatic, Antihypertensive effect and antihuman retrovirus activities and for *V.vinifera*like anticancer, protect cardiovascular health, glucose balance. Habitat of *S .rebaudiana* is originating from tropical America, it can be found in the wild in Paraguay, Brazil and Argentines in sunny locations with sandy acidic soils and habitat of *V.vinifera*is originating from tropical and subtropical climates, some occurs temperate habitat. Currently Japan and Korea are the largest market of stevia. *S. rebaudiana Bretoni* is also being cultivated by Indian farmers, since last decade and used as a dry leaf or as a proceed sweetener.

Materials and Method

Stevia and Grape plant:-

Stevia plants **(***Stevia rebaudiana*) were collected from Agriculture University, Department of Plant tissue culture, Aanand, India and Grapes plants ***(Vitis vinifera L.)*** were collected from Shittal Nursery, Satelliteof Ahmadabad.

Reagent:-

Organic solvents like ethanol, ethyl acetate, chloroform, methanol;

Spraying reagent like 5% methanolic sulfuric acid,10% ethanol sulfuric acid.

Preparation Stevia leaves and Grape leaves powder:-

Fresh leaves were removed from the plant then washed in clean water and spread on filter paper or tray. Leaves were dried under shed at room temperature for 24-48 hrs. Dry leaves were packed in polythene bags and stored at freeze condition until used. By using mortar fine powder of leaves were prepared. The dry leaves were used for extraction preparation.

Preparation of extraction:-

For Stevioside extraction,0**.**1g of dried leaves of stevia rebaudiana powder. Solvent extract with ethanol (11.6ml) and water (9.4 ml) in earlynmayer flask by shaking for 30 minute in 70°Cwater bath. After the extracts were cooled and it was filtered by use of whatman filter paper no.1and evaporated to dryness 30°C at room temperature. The residue is dissolved in 0.1ml ethanol.

For Resveratrol extraction,1g dried leaves of grape plant powder or callus. Solvent extract with 95% ethanol (7ml) for 2hrs, filtered and evaporated to dryness 30°C at room temperature. The residue is dissolved in 1ml ethanol

Above both extraction was used for analyzed for TLC

Thin layer chromatography (TLC):-

TLC was performed under percolated silica gel (5cm×10cm) plate of uniform thickness (0.2mm).Spots of both samples were applied on the plate by the use of capillary tube.ForStevioside [GurpreetKauret all (2014)]**,** Solvent system in chamber was saturated with mobile phase

chloroform: methanol: water (6.9:2.6:0.5) and For resveratrol,Solvent system in chamber was saturated with mobile phase ethyl acetate: methanol: water (10: 1.3: 1) .The mobile phase allowed to run up to a distance of 8cm from the base. The plate was removed and air dried for 10minute. The spot was visualized by spraying the solution of 5% methanol sulfuric acid and 10% ethanol sulfuric acid on plate followed by heating the plate at 110 C for 10 minute respectively. Let the plate allowed cool and look the spot on the plate. Write down the result.

Result

The ethanolic extraction of stevia and grape leaf extract exhibited one component could be identified with R_f values 0.65 and 0.90 on thin layer chromatographic plate [Silica gel plate, solvent system; For stevioside, chloroform: methanol: water in the ratio 6.9:2.6:0.5 (V/V/V)] and [Solvent system; For resveratrol, ethyl acetate:methanol:water in the ratio 10:1.3:1 (V/V/V)].This spot was visualized by 5% methanol sulfuric acid and 10% ethanol sulfuric acid reagent followed by heating at 110°C for 10 minutes and spots showed black coloration.

Table 1: Characteristics of Stevioside and Resveratrol of stevia leaf extract and grape leaf extract (Ethanolic extract) on thin layer chromatographic plate.

No. of sample	R_f Values	Colour in atmosphere	Colour after spraying with 5% methanol sulphuric acid reagent and heating 110 °C, 10min	Name of components
1. (standard)	0.63	Yellowish brown	Black	Stevioside
2. (Stevia leaf extract)	0.65	Yellowish brown	Black	Stevioside
3. (standard)	0.87	Yellowish brown	Black	Resveratrol
4. (Grape leaf extract)	0.90	Yellowish brown	Black	Resveratrol

$$\text{Retardation factor (RF)} = \frac{\text{Distance travelled by Solute}}{\text{Distance travelled by solvent}}$$

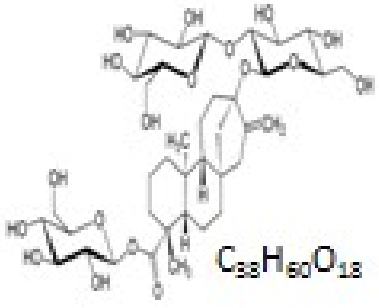
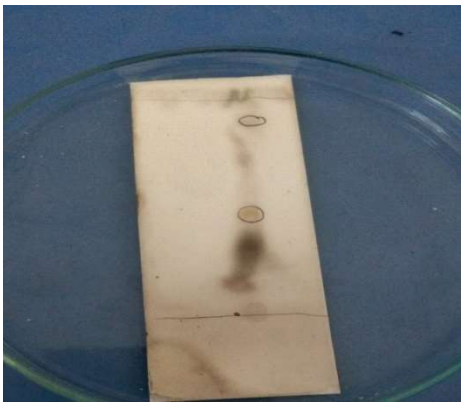


Fig 1: Spots of Stevioside on TLC plate and Structure of Stevioside



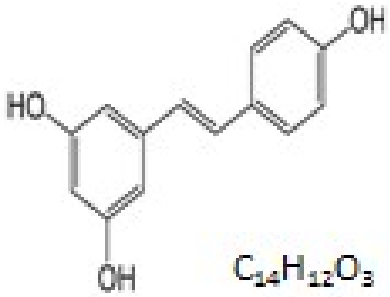


Fig 2: Spots of Resveratrol on TLC plate and structure of Resveratrol.

Discussion

*Stevia rebaudiana*and *Vitisvinifera*were medicinal plants were taken and the polyphenol compound like Stevioside from *S.rebaudiana* and Resveratrol from *V.vinifera* were detected by thin layer chromatography using solvents such as chloroform: methanol: water and ethyl acetate: methanol:water for *S.rebaudiana*and *V.vinifera* respectively and their R_f value was 0.65 Stevioside and Resveratol 0.90 was obtained which was similar to the standard R_f values.

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Aknowledgement

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